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Abstracts

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Oral Session 2: Hepatitis Viruses *Chairs: Johan Neyts, Ph.D. and Angela Lam, Ph.D.* 1:30–4:00 pm Sofia 1 and 2

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IDX375, A Novel Allosteric HCV Polymerase Inhibitor: *In Vitro* Antiviral Activity and Preclinical Profile

C.B. Dousson[1,*], S.S. Good[2], M. La Colla[2], J. Bilello[2], M. Seifer[2], D.N. Standring[2], J.-L. Paparin[1], D. Surleraux[1]

[1] *Idenix Pharmaceuticals, Parc Euromedecine, France*
[2] *Idenix Pharmaceuticals, Inc., Cambridge, USA*

Background: The HCV NS5B polymerase is essential for HCV replication and has four distinct non-nucleoside allosteric binding sites that provide clinically validated HCV targets. This report presents the characterization of IDX375, a novel non-nucleoside inhibitor (NNI) that binds to the palm (NNI3) site domain of NS5B.

Methods: The antiviral activity and specificity of IDX375 were assessed in standard assays utilizing purified polymerases and HCV replicons. The pharmacokinetic profile of IDX375 was determined by standard methods in mice and cynomolgus monkeys.

Results: An extensive evaluation of five structurally diverse series of palm inhibitors led to the selection of IDX375 as the clinical candidate. IDX375 exhibited genotype 1b and 1a enzymatic potencies of 5 and 16 nM, respectively, with an EC_{50} of 2.3 nM in the 1b HCV replicon, and was inactive against human cellular polymerases. IDX375 generally showed additivity in combination with the HCV PI IDX320 or the nucleotide IDX184, but exhibited very strong synergy when combined with both classes of direct acting antiviral agents. Substantial plasma exposures (μM C_{max} levels) were attained following oral administration of IDX375 (15 mg/(kg day)) in the mouse and cynomolgus monkey, and IDX375 was substantially concentrated in the liver in the mouse.

Conclusions: IDX375 is a potent inhibitor of the HCV polymerase that has been dosed in healthy volunteers (de Bruijne et al., in press) and is currently in a 3-day proof of concept study in HCV-infected subjects. The current pharmacokinetic profile of IDX375 in human subjects supports a twice-daily dosing regimen.

Reference

de Bruijne, J., Wetering de Rooij, J.v.d., Leempoels, J., Zhou, X.J., Weegink, C.J., Molenkamp, R., Schinkel, C.J., Temam, M.F., Molles, J., Chen, J., Pietropaolo, K., Sullivan-Bólyai, J.Z., Mayers, D., Reesink, H.W., in press. Phase I Study in Healthy Volunteers and Patients with IDX375, a Novel Non-Nucleoside HCV Polymerase Inhibitor.

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Study of NS5B Oligomerization by FRET: Characterization and Inhibition

Itxaso Bellón-Echeverría[1,*], Alberto J. López-Jiménez[1,2], Pilar Clemente-Casares[1], Jose A. Encinar[4], Elisa Martínez-Alfaro[2], Ricardo Pérez-Flores[3], Antonio Mas[1]

[1] *Centro Regional de Investigaciones Biomédicas (CRIB), Albacete, Spain*
[2] *Disease Infectious Unit, Complejo Hospitalario Universitario de Albacete, Albacete, Spain*
[3] *Digestive Department, Complejo Hospitalario Universitario de Albacete, Albacete, Spain*
[4] *Instituto de Biología Molecular y Celular, Universidad Miguel Hernandez, Elche, Spain*

Hepatitis C virus (HCV) is a positive strand RNA virus ((+)RNA) that replicates its genome in replication complexes (RC) associated to endoplasmic reticulum (ER)-derived micro-vesicles. One key protein in these complexes is NS5B, the viral RNA-dependent RNA-polymerase (RdRp). Recently, it has been demonstrated that NS5B interacts itself forming oligomers, and mutations that disrupt these interactions are lethal for polymerase function. Therefore, NS5B oligomerization could be a new target for the design of anti-HCV compounds. We have developed a new accurate method to analyze NS5B–NS5B interactions by using Förster-resonance-energy-transfer (FRET) *in vitro* using recombinant proteins. This method has allowed us to analyze the conditions driving the interactions between NS5B polymerases, in our case the NS5B–cyan and NS5B–citrine constructs. The most important interactions among monomers are electrostatic because of the dependence on ionic strength. Both, NaCl and KCl lead to concentration-dependent changes in the oligomerization status of NS5B. We have also tested different combinations of point mutants affecting FRET values from zero to around 100%. Cooperativity in RNA synthesis activity has also been analyzed by determining the Hill coefficient and the results are consistent with those obtained for oligomerization. We have extended these studies to HCV RNA-polymerases from different genotypes (genotypes 1–5), including the analyses of reaction